INFLUENCE OF EARTHWORMS ON THE NUMBER OF BACTERIA IN THE SOIL

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Publications on the influence of earthworms on the bacterial population of the soil show many controversial results. An increase in the number of bacteria in earthworm excrements compared with the original soil was found by BASALIK (1913), STÖCKLI (1928), RUSCHMANN (1953), SCHÜTZ et al. (1956). DAY (1950) found either an increase or a decrease in the number of bacteria in the earthworm excrements compared with those of the original soil. BRÜSEWITZ (1959) started to work with soil mixed with glucose or plant material. In the worm excrements of soil mixed with glucose the number of bacteria was less than in the soil – glucose mixture, whilst in the excrements of soil mixed with plant material more bacteria were found in the excrements than in the soil – plant material mixture.

In the soil the readily available glucose will be broken down by the microorganisms. Since no new food is added in the alimentary canal of the worms and even bacteria might be digested there, less bacteria can be expected in the excrements. The plant material mixed with the soil does not disappear quickly and will be disintegrated in the alimentary canal of the worms and therefore will even give a better mixture for bacterial development in the excrements.

To analyse the controversial results of the above publications, different species of earthworms were placed in jars filled with clay soil and with shed alder leaves on top. These leaves were readily eaten by the earthworms. The species of earthworms used in these experiments were: *Allolobophora chlorotica*, *A. rosea*, *A. caliginosa*, *A. longa*, *Lumbricus terrestris*, all earth inhabiting worms which have an influence on the structure of the soil and *Eisenia foetida* which is not soil inhabiting but lives in compost or manure. As this last species has been used by most of the investigators working with earthworms it was included in this experiment. *Allolobophora chlorotica*, *A. rosea* and *A. caliginosa* live in the upper layers of the soil and deposit their excre-

ments in the soil. *Lumbricus terrestris* and especially *Allolobophora longa* are deep living worms depositing their excrements on the surface of the soil.

Plate counts were made on soil extract agar mixed with 0.05 % K_2HPO_4 (LÖHNIS, 1920), giving excellent results. A large number of bacteria as well as actinomycetes could be counted and spreading bacteria were more suppressed than on Czapek agar or pepton agar. More actinomycetes could be counted than on the medium advised by PORTER et al. (1960).

The soil or the excrements were mixed for 15 minutes in a household mixer. This gave better results than mixing with a magnetic stirrer or vibromix.

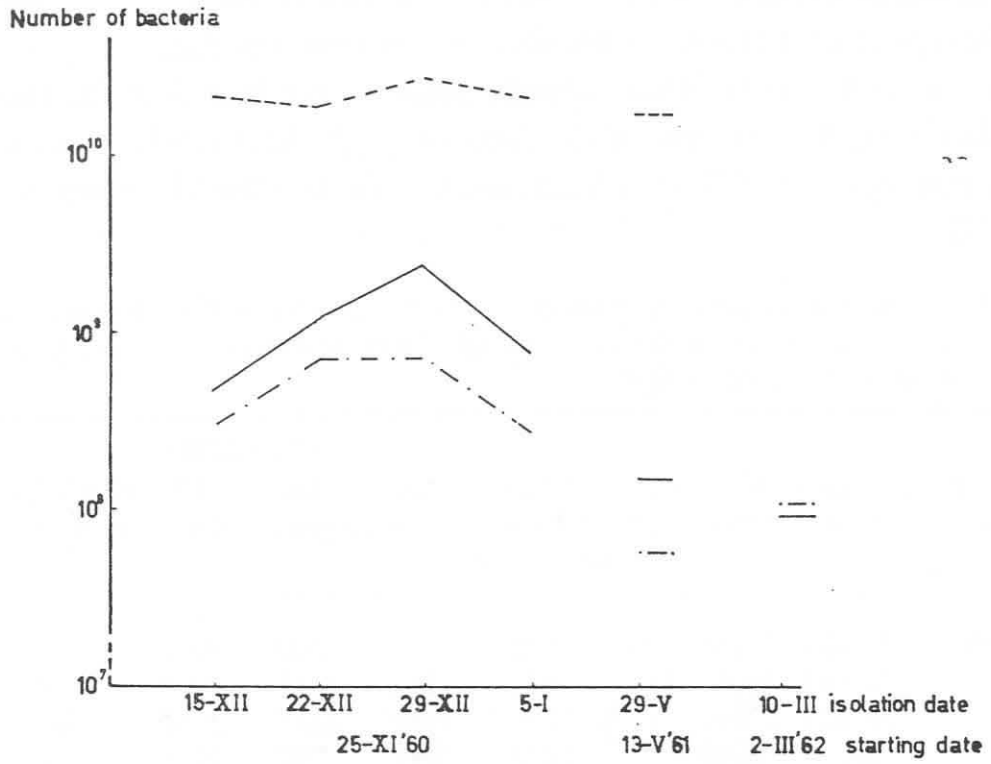

Fig. 1. Number of bacteria in alder leaves, clay soil and excrements of *Allolobophora caliginosa*.
— . — clay soil
——— excrements
- - - - alder leaves

Fig. 1 gives the bacterial counts of clay soil, alder leaves and the excrements of *Allolobophora caliginosa* fed on the clay soil covered with alder leaves. The counts from the alder leaves and the clay soil were made from control jars without earthworms.

From different jars started on 25-XI-'60 counts were made after 3, 4, 5 and 6 weeks. From the experiment started on 13-V-'61 counts were made after two weeks. Three or four earthworms were used in each jar and ex-

crements of all ages were collected from the soil in the jars. In the experiment started on 2-III-'62 the earthworms were put in washed clay soil without any addition of leaves. In the washing all large particles of organic matter were removed. On the advice of Dr. Doeksen the earthworms were kept on filter-paper and nylon sponges drenched with an inorganic salt solution until their digestive tracts were empty. After feeding for 6 days on the clay soil they were washed, dried with filter paper and put in clean sterile containers. Here for two days the deposited excrements were collected. Plate counts were made simultaneously from the washed clay and from the excrements.

In both the experiments where the earthworms mixed the clay with the alder leaves the number of bacteria in the excrements was higher than in the clay soil but much lower than in the alder leaves. Without the addition of the alder leaves almost the same number of bacteria was found in the clay soil and in the excrements. These results point to the fact that the increase in bacteria during the passage of the food through the digestive tract is caused by the mixing of the different fractions, in this case the alder leaves with the clay soil.

TABLE 1. Number of bacteria in millions in the excrements of 6 earthworm species and in the leaves and in the earth out of which the excrements are formed, calculated per gram dry weight

Starting date	Isolation date	Alder leaves	Clay soil	Excrements					
				Allolobophora chlorotica	*All. rosea*	*All. caliginosa*	*All. longa*	*Lumbricus terrestris*	*Eisenia foetida*
25-XI-1960	15-XII	22169	296	725		500	4630	742	
,, ,,	22-XII	19081	682	1031	1292	1142	1057	1777	
,,	29-XII	27567	711	2246	1910	2357	3252	4243	
,,	5-I	20964	277	1233	1041	790	3074	2071	
22-I-1961	16-II	6847	55				2014		
13-V-1961 [1])	29-V	23183	1140			2818		2887	
16-II-1962	18-II	18675	89						1437
2-III-1962	14-III		88			85			

[1]) In this experiment the actinomycetes were included in the counts. The percentage actinomycetes in the other experiments varied from 1 %—30 %.

All the other earthworm species used in these experiments gave the same results (table 1). Even *Eisenia foetida* with its different mode of living gave the same results when placed in the jars with clay soil and alder leaves.

In only one experiment, which is not included in table 1 were the results

more variable. This was probably due to differences in the circumstances under which the jars were kept, since it were especially the alder leaves on top of the control jars which gave variable results.

All these laboratory experiments were started to compare them with a field experiment in one of our newly reclaimed polders. In these polders no earthworms can be found after drainage.

The site of the experiment is an apple orchard under grass in the "Noord-Oost Polder". This orchard is subdivided into different sections by poplars. In 1947 two centres in section I were inoculated with earthworms, mainly *Allolobophora caliginosa*. Since then the inoculated and the neighbouring section (section I and II) of the orchard have been invaded by these earthworms. Section I was ploughed in 1960, section II and III were left undisturbed. Section III has not yet been invaded by the earthworms. The earthworm countings were performed by Dr. J. A. van Rhee.

The grass in this orchard was mulched and this resulted, especially in section III in a brown mor-like layer underneath the grass. This same layer

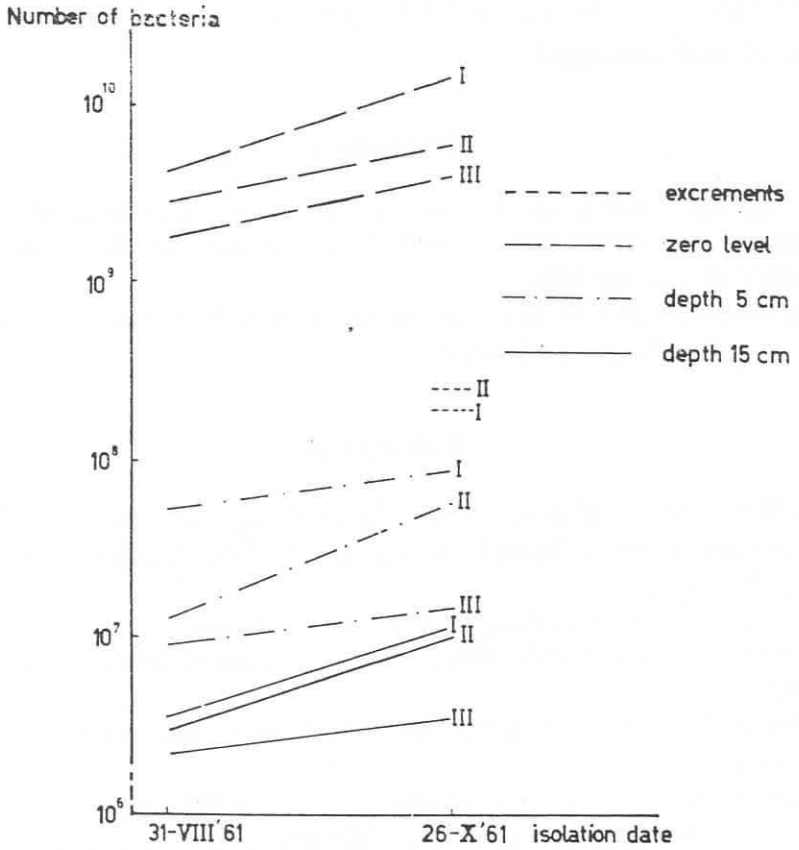

Fig. 2. Number of bacteria in the soil and in earthworm excrements in an apple orchard in the "Noord Oost Polder".
I: ploughed, earthworms present. II: undisturbed, earthworms present. III: undisturbed, no earthworms.

but much thinner could be found underneath the grass in section II, but no such layer could be found in section I.

Plate counts of bacteria (Fig. 2) were made from this mor-like top layer (scratched off grasslayer from section I), from the 5 and 15 cm layer of the clay soil and from the excrements of the earthworms. Here also the number of bacteria in the excrements was in between the number of the toplayer and of the clay soil.

From all these experiments it is evident that the mixing of clay soil and top layer or mulched grass increases the number of bacteria in excrements as compared with the soil. This corresponds well with the results of Brüsewitz and explains why other investigators who compared only the excrements with the soil and did not take into account the organic matter mixed by the worms with the subsoil, found an increase of bacteria in the excrements.

These results do not exclude the possibility that special groups of bacteria may decrease or increase in the intestine of the worms.

In Fig. 2 the number of bacteria in section I of the orchard is always higher than in section II and there again the number is higher than in section III. This might be caused by the mixing of the toplayer with the subsoil by the earthworms in parts I and II and the ploughing of part I. Since no bacterial counts were made in this field before the earthworms were brought in, this evidence is not conclusive.

Summary

The increase in the number of bacteria in the excrements of earthworms compared with those of the soil in which these worms live, is caused by their mixing the soil with the organic matter of the top layer.

There is an indication that in the field the number of bacteria in soil with earthworms is higher than in soil without earthworms.

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Discussion

J. E. SATCHELL: The leaves of alder are exceptional in their high nitrogen content. Would Dr. Went expect similar results from leaves such as birch or hazel with a higher C/N ratio?
Miss J. C. WENT: These leaves have not been used. Therefore no results can be told. Grass leaves which probably have a high nitrogen content also gave similar results.
G. ZACHARIAE: Es ist sehr schwer die Ergebnisse der Versuche auf das Freiland zu übertragen. Im Experiment wurde Erlenlaub benutzt, die Wirtschaftwälder bestehen aber aus Buchen, Eichen und Nadelhölzern. Ist das Futtermaterial nicht schon maximal mikrobiel besiedelt?
Miss J. C. WENT: In diesem Falle wäre keine Erhöhung der Mikrobenzahl bei der Passage durch den Darm des Regenwurmes zu erwarten.
J. DOEKSEN: Total bacterial counts may be misleading as it is quite possible that certain groups of bacteria are digested while others are stimulated, especially as the symbiontic bacteria from the nephridia, which are also present all over the earthworms, are able to produce antibiotics which might kill at least some of the groups of ingested microorganisms. I realise that it is asked too much from the microbiologists to make their counts on a variety of different plates but in my opinion it will be the only way to shed more light on these controversial problems
Mme C. JEANSON-LUUSINANG: Les méthodes de culture utilisées en microbiologie ne sont pas suffisamment sensibles, elles peuvent seulement indiquer le sens du phénomène. Le comptage direct, plus long et plus délicat, permettrait peut-être d'obtenir des résultats plus précis tant pour l'étude de la microflore totale que pour celle de chaque groupe de microorganismes.